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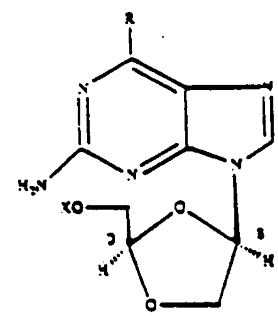
(57) Abstract

The invention is a method for the treatment of humans infected with HBV that includes administering an HBV treatment amount of an enatiomerically pure β-D-dioxolanyl purine nucleoside of formula (I), wherein R is OH, Cl, NH_2 or H, and X is selected from the group consisting of alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt.



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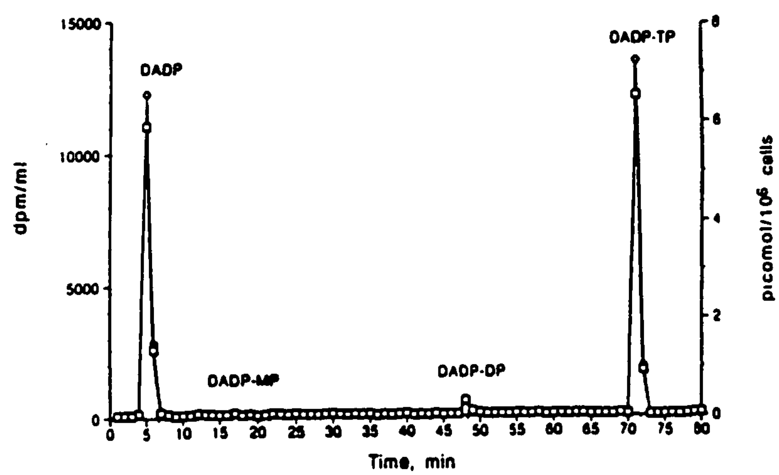


ENANTIOMERICALLY PURE β-D-DIOXOLANE NUCLEOSIDES WITH SELECTIVE ANTI-HEPATITIS B VIRUS ACTIVITY

Background of the Invention

This invention is in the area of methods for the treatment of hepatitis B virus (also referred to as "HBV") that includes administering an effective amount of one or more of the active compounds disclosed herein, or a pharmaceutically acceptable derivative or prodrug of one of these compounds.

HBV is second only to tobacco as a cause of human cancer. The mechanism by which HBV induces cancer is unknown, although it is postulated that it may directly trigger tumor development, or indirectly trigger tumor development through chronic inflammation, cirrhosis, and cell regeneration associated with the infection.

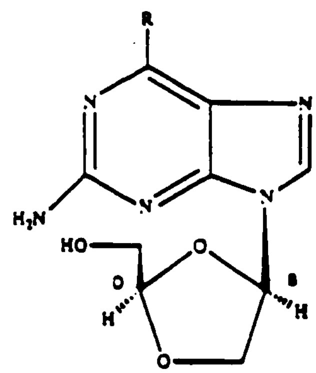
Hepatitis B virus has reached epidemic levels worldwide. After a two to six month incubation period in which the host is unaware of the infection, HBV infection can lead to acute hepatitis and liver damage, that causes abdominal pain, jaundice, and elevated blood levels of certain enzymes. HBV can cause fulminant hepatitis, a rapidly progressive, often fatal form of the disease in which massive sections of the liver are destroyed.

Patients typically recover from acute viral hepatitis. In some patients, however, high levels of viral antigen persist in the blood for an extended, or indefinite, period, causing a chronic infection. Chronic infections can lead to chronic persistent hepatitis. Patients infected with chronic persist nt HBV are m st c mm n in developing countries. By mid-1991, there were approximat ly 225 million chronic carriers f HBV in Asia alone, and w rldwide, alm st 300 milli n

the tr atment of human patients or other hosts infected with HBV.

Summary of the Invention

In a preferred embodiment, the invention is a method for the treatment of humans infected with HBV that includes administering an HBV treatment amount of an enantiomerically pure β-D-dioxolanyl purine nucleoside of the formula:



wherein R is OH, Cl, NH2. or H, or a pharmaceutically acceptable salt or derivative of the compound, optionally in a pharmaceutically acceptable carrier or diluent. The compound wherein R is chloro is referred to as (-)-(2R,4R)-2-amino-6-chloro-9-[(2-hydroxymethyl)-I,3-dioxolan-4-yl]purine. The compound wherein R is hydroxy is (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine. The compound wherein R is amino is (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine. The compound wherein R is hydrogen is (-)-(2R,4R)-2-amino-

containing these compounds are useful in the prevention and treatment of HBV infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV antibody or HBV-antigen positive or who have been 5 exposed to HBV.

In one embodiment of the invention, one or more of the active compounds is administered in an alternative fashion with one or more other anti-HBV agents, to provide effective anti-HBV treatment. Examples of anti-HBV agents that can be used in alternation therapy include but are not limited to the enantismer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane ("FTC", see W092/14743), its physiologically acceptable derivative, or physiologically acceptable salt; (-)-enantismer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane (also referred to as "BCH-189" or 3TC, see EPA Publication No. 0 382 526), its physiologically acceptable derivative, or physiologically acceptable salt; an enantismer or racemic mixture of 2'-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2'-fluoro-5-ethyl-arabinosyluracil (FEAU); carbovir, or interferon.

Any method of alternation can be used that provides treatment to the patient. Nonlimiting examples of alternation patterns include 1-6 weeks f administration of an effective amount f one agent followed by 1-6 weeks of administration of an

-1,3-dioxolan-4-yl]purine; DG, (-)-(2R,4R)-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]guanine; DAPD, (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine).

Figure 3 is a graph of the effect of purine dioxolanes and AZT on colony formation of human granulocyte-macrophage precursor cells, as measured in terms of percent of cells of control versus the log of the concentration of test drug. For abreviations used, see description of Figure 2.

Figure 3 is a graph of the percent inhibition of HBV DNA replication in 2.2.15 cells on day 9 in varying concentrations of test compounds. For abreviations used, see description of Figure 2 ((-)-FTC is (-)-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane). See Table * for corresponding data.

Figure 4 is a graph of the uptake of 5 μM of tritiated (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine (DAPD) in Hep2G cells. Extract was obtained at four hours after exposing cells to DAPD. 1000dmp/pmol; 80 μL injected.

Figure 5 is a graph of the uptake of 5 μM of tritiated (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine (DAPD) in Hep2G cells. Extract was obtained at twelve hours after exposing cells to DAPD. 1000dmp/pmol; 145 μL injected.

Detailed Description of the invention

As used herein, the term "enantiomerically pure" refers to a nucleoside composition that includes at least approximately 95%, and preferably 97%, of a single enantiomer of that nucleoside.

The invention as disclosed herein is a method and composition for the treatment of HBV infection, in humans or other host animals, that includes

As used herein, the term alkyl specifically includes but is not limited to methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, t-butyl, isopentyl, amyl, t-pentyl, cyclopentyl, and cyclohexyl. As used herein, the term acyl specifically includes but is not limited to acetyl, propionyl, butyryl, pentanoyl, 3-methylbutyryl, hydrogen succinate, 3-chlorobenzoate, benzoyl, acetyl, pivaloyl, mesylate,propionyl, valeryl, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic. The nucleoside can also be provided as a 5' ether lipid, as disclosed in the following references, which are incorporated by reference herein: Kucera, L.S., N. Lyer, E. Leake, A. Raben, Modest E.J., D. L.W., and C. Piantadosi. 1990. Novel membrane-interactive ether lipid analogs that inhibit infectious HIV-1 production and induce defective virus formation. AIDS Res Hum Retroviruses. 6:491-501; Piantadosi, C., J. Marasco C.J., S.L. morris-Natschke, K.L. Meyer, F. Gumus, J.R. Surles, K.S. Ishaq, L.S. Kucera, N. lyer, C.A. Wallen, S. Piantadosi, and E.J. Modest. 1991-Synthesis and evaluation of novel ether lipid nucleoside conjugates for anti-HIV activity. J Med Chem. 34:1408-1414; Hostetler, K.Y., D.D. Richman, D.A. Carson, L.M. Stuhmiller, G.M. T. van Wijk, and H. van den Bosch. 1992. Greatly enhanced inhibition of human immunodeficiency virus type 1 replication in CEM and HT4-6C cells by 31-deoxythymidine diphosphate dimyristoylglycerol, a lipid prodrug of 31-deoxythymidine. Antimicrob Agents Chemother. 36:2025-2029; Hostetler, K.Y., L.M. Stuhmiller, H.B. Lenting, H. van den Bosch, and D.D. Richman. 1990. Synthesis and antiretroviral activity of phospholipid analogs of

Modifications of th active compound, specifically at the N^6 and 5'-O positions, can affect the bioavailability and rate of metabolism of the active species, thus providing control over the delivery of the active species.

The active compound, or pharmaceutically acceptable derivative or salt thereof can also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, or other antivirals, including anti-HBV or anti-HIV agents.

I. Preparation of Enantiomerically Pure Dioxolane Nucleosides

Enantiomerically pure ß-D-dioxolane-nucleosides can be prepared as disclosed in detail below, and as described in PCT/US91/09124. The process involves the initial preparation of (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-ox-ymethyl)-dioxolane from 1,6-anhydromannose, a sugar that contains all of the necessary stereochemistry for the enantiomerically pure final product, including the correct diastereomeric configuration about the 1 position of the sugar (that becomes the 4'-position in the later formed nucleoside).

The (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane is condensed with a desired heterocyclic base in the presence of $SnCl_4$, other Lewis acid, or trimethylsilyl triflate in an organic solvent such as dichloroethane, acetonitrile, or methylene chloride, to provide the stereochemically pure dioxolane-nucleoside.

In preparing enantiomerically pure dioxolane nucleosides, care should be taken to avoid strong acidic c nditions that would cl ave the dioxolane ring. Reactions should be performed, if possible,

n-Bu_4NF in good yields. The α-isomers **12** and **13** were prepared by the similar procedure as the β-isomers.

Example 1 Preparation of Enantiomerically Pure β-D-Dioxolanyl Purine Nualeosides

(2R,4R) and (2R,4S)-9-[[2-[(tert-Butyldiphenylsilyl) oxylmethyl]- 1,3-dioxolan-4-yl]-6-chloro-2-fluoropurine (2 and 3).

A mixture of 2-fluoro-6-chloropurine (4.05 g, 23.47 mmol) and ammonium sulfate (catalytic amount) in hexamethyldisilazane (940 mL) was refluxed for 2 hours. The resulting solution was concentrated under anhydrous conditions to yield silylated 2-fluoro-6-chloropurine as a white solid. To a cooled (OOC) and stirred solution of silylated 2-fluoro-6-chloropurine (5.69 g, 23.69 mmol) and compound 1 (7.84 g, 19.57 mmol) in dry methylene chloride (175 mi) was added TMSOTF (4.41 mL, 23.44 mmol). The reaction mixture was warmed to room temperature and stirred for 16 hours, during which time, all the initially formed N_7 condensed product was converted to N_9-isomer. The reaction mixture was quenched with saturated $NaHCO_3$ solution (50 mi) and stirred for an additional 20 minutes at room temperature, evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (200 mi), washed with water and brine, dried (anhydrous Na_2SO_4), filtered and evaporated to give a solid residue, which was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford a mixture of β-anomer 8 and α-anomer 9 (1.3:1; β/α) as a white crystalline solid (6.30 g, 62.8%). The analytical sample was purified by preparative TLC using CH_2CL_2-acetone (19:1) as the 5 devel ping system to give 2 (R_f= 0.50) and 3 (R_f =

(-)-(2R,4R)-2-Flu r -9-[(2-hydr xymethyl)-1,3-di x lan-4- yl]adenine (11).

A solution of 5 (0.56 g, 1.12 mmol) in THF (20 mL) was treated with 1 M *n*-Bu_4NF/THF (1.35 mL, 1.35 mmol) to furnish 22 (0.24 g, 85%) as a white crystalline solid, which was recrystallized from MeOH: UV (H_2O) λ_{max} 260.8 nm (ε17,010), 268.5 (sh) nm (ε13,510) (pH 7), 261. 0 (ε16,390), 268.5 (sh) (ε13,300) (pH 2), 260.8 (ε16,700), 268.5 (sh) (ε13,200) (pH 11). Anal. ($C_9H_{10}FN_5O_3$) C, H, F, N.

(-)-(2R,4R)-9-[(2-Hydroxymethyl)-1,3-dioxolan-4-yl] guanine (14).

A mixture of 4 (0.29 g, 0. 57 mmol), $HSCH_2CH_2OH$ (0.51 mL) and 1.0 M NaOMe/MeOH (11.5 mL) in MeOH (20 mL) was refluxed for 3 hours. The reaction mixture was cooled and neutralized with glacial acetic acid. The solution was evaporated to dryness, and then the residue was triturated with $CHCI_3$, filtered and the filtrate was taken to dryness to give crude compound 8 (0.21 g, 75%), which without further purification was subjected to desilylation to give compound 3 (0.07 g, 61%) as a microcrystalline solid, which was recrystallized from MeOH: UV (H_2O) λ_{max} 252.0 (ε8,730) (pH 7), 254.4 (ε12,130), 277.5 (sh) (ε8,070) (pH 2), 264.3 (ε10,800) (pH11). Anal. ($C_9H_{11}N_5O_4$) C, H, N.

(-)-(2R,4R)-2-Amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine (15).

A steel bomb was charged with compound 4 (0.28 g, 0.55 mmol), anhydrous ethanol (20 mL) saturated with NH_3, and heated at 90°C for 6 hours. After cooling, the compound 9 (0.26 g, 95%) obtained on evaporated of the solvent *in vacuo*, and then desilylated according to the same procedure described for preparati n of 12 to give 15 (0.10 g,

depressions greater than 3.0-fold (for HBV virion DNA) or 2.5-fold (for HBV DNA replication intermediates) from the average levels for these HBV DNA forms in untreated cells are generally considered to be statistically significant [$P<0.05$] (Korba and Gerin, Antiviral Res. 19: 55-70, 1992). The levels of integrated HBV DNA in each cellular DNA preparation (which remain constant on a per cell basis in these experiments) were used to calculate the levels of intracellular HBV DNA forms, thereby eliminating technical variations inherent in the blot hybridization assays.

Typical values for extracellular HBV virion DNA in untreated cells range from 50 to 150 pg/ml culture medium (average of approximately 76 pg/ml). Intracellular HBV DNA replication intermediates in untreated cells range from 50 to 100 pg/ug cell DNA (average approximately 74 pg/ug cell DNA). In general, depressions in the levels of intracellular HBV DNA due to treatment with antiviral compounds are less pronounced, and occur more slowly, than depressions in the levels of HBV virion DNA.

For reference, the manner in which the hybridization analyses were performed for these experiments results in an equivalence of approximately 1.0 pg intracellular HBV DNA/ug cellular DNA to 2-3 genomic copies per cell and 1.0 pg of extracellular HBV DNA/ml culture medium to 3 x 10^5 viral particles/ml.

Toxicity analyses were performed in order to assess whether any observed antiviral effects are due to a general effect on cell viability. The method used was based on the uptake of neutral red dye, a standard and widely used assay for cell viability in a variety of virus-host systems, including HSV (herpes simplex virus) and HIV.

Toxicity analyses were performed in 96-well flat bottomed tissue culture plates. Cells for the toxicity analyses were cultured and treated with test compounds with the same schedule as used for the antiviral evaluations. Each compound was tested at 4 concentrations, each in triplicate cultures. Uptake of neutral red dye was used to determine the relative level of toxicity. The absorbance of internalized dye at 510 nM (A_{510}) was used for the quantitative analysis. Values are presented as a percentage of the average A_{510} values (+/- standard deviations) in 9 separate cultures of untreated cells maintained on the same 96-well plate as the test compounds. The percentage of dye uptake in the 9 control cultures on plate 40 was 100 +/- 3. At 150-190 μM 2',3'-ddC, a 2-fold reduction in dye uptake (versus the levels observed in untreated cultures) is typically observed in these assays (Korba and Gerin, Antiviral Res. 19: 55-70, 1992).

Example 3 Anti-Hepatitis B Virus Activity

As indicated in Table 2, within normal variations, levels of HBV virion DNA and intracellular HBV replication intermediates [HBV RI] remained constant in the untreated cells over the challenge period. The positive treatment control, 2',3'-dideoxycytosine [2',3'-ddC], induced significant depressions of HBV DNA replication at the concentration used. Previous studies have indicated that 9-12 μM 2',3'-ddC, a 90% depression of HBV RI (relative to average levels in untreated cells) is typically observed in this assay system (Korba and Gerin, Antiviral Res. 19: 55-70, 1992).

All three test compounds were potent inhibitors of HBV replication, causing depression of HBV virion DNA and HBV RI to a degree comparable to, or greater than, that observed following treatment with 2',3'-ddC.

1.0 μM (-)- -2-NH2-6-Cl-purine-dioxolane		66	59	12	0	1.2	3
		70	45	10	1	1.4	3
		74	56	15	0	0.9	1
		61	43	11	0	1.1	2
	Mean	67.75	50.75	12.00	0.25	1.15	2.25
	S.D.	5.56	7.93	2.16	0.50	0.21	0.96
	% inhibition	-7.54	21.62	84.36	99.66	54.46	96.94
1.0 μM (-)- -2-NH2-6-Cl-purine-dioxolane		52	67	28	5	2.3	14
		58	59	34	6	2.4	11
		64	59	35	9	2.6	13
		77	62	26	8	2.1	10
	Mean	62.75	61.75	30.75	7.00	2.35	12.00
	S.D.	10.69	3.77	4.43	1.83	0.21	1.83
	% inhibition	0.40	4.63	59.93	90.60	6.93	83.67
1.0 μM (-)- -2-NH2-6-Cl-purine-dioxolane		70	86	22	2	2.0	6
		50	59	24	4	1.9	6
		56	56	23	2	1.4	3
		73	62	20	3	2.1	4
	Mean	62.25	66.75	22.25	2.75	1.85	4.75
	S.D.	113.72	1.71	0.96	0.31	1.50	
	% inhibition	1.19	-1.54	71.01	96.31	26.73	93.54
1.0 μM (-)- -2-NH2-6-Cl-purine-dioxolane		51	77	60	18	2.0	28
		59	62	70	12	2.2	23
		74	73	69	14	2.8	25
		67	61	82	11	2.4	20
	Mean	62.75	68.25	70.25	13.75	2.35	24.00
	S.D.	9.95	7.97	9.03	3.10	0.34	3.37
	% inhibition	0.40	-5.41	8.47	81.54	6.93	67.35

* Analysis of intracellular HBV DNA was 24 hours following the 9th day of treatment. DNA in each cell DNA preparation were used o calculate the levels of episomal 3.2kB HBV genomes (MONO.) and HBV DNA replication intermediates (RI).

** A "zero" indicates an und tectabl level of HBV DNA, sensitivity cutoff was 0.1 pg[ml].

RI EC_{50} and EC_{90}, cytotoxicity and selectivity index for DG, DAPG, ACPD, FTC, and DDC.

Exampl 6

Figure 5 is a graph of the uptake of 5 μM of tritiated (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine (DAPD) in HepG2 cells. Extract was obtained at four hours after exposing cells to DAPD (1000 dmp/pmol; 80 μL injected). The data indicates that the compound is primarily metabolised intracellularly to the triphosphate form.

Example 7

Figure 6 is a graph of the uptake of 5 μM of tritiated (-)-(2R,4R)-2-amino-9-[(2-hydroxymethyl)-1,3-dioxolan-4-yl]adenine (DAPD) in HepG2 cells. Extract was obtained at twelve hours after exposing cells to DAPD (1000 dmp/pmol; 145 μL injected). The data indicates that after four hours of incubation with the tritiated compound, there are high intracellular levels of the triphosphate.

IV. Preparation of Pharmaceutical Compositions

The compounds disclosed herein and their pharmaceutically acceptable salts, prodrugs, and derivatives, are useful in the prevention and treatment of HBV infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV antibody or HBV-antigen positive or who have been exposed to HBV.

70 to 1400 mg of active ingredient per unit dosage form. A oral dosage of 50-1000 mg is usually convenient.

Ideally the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.2 to 70 μM, preferably about 1.0 to 10 μM. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or administered as a bolus of the active ingredient. The concentration of active compound in the drug composition will depend on absorpeion, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. The active ingredient may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

A preferred mode of administration of the active compound is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, r capsules.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

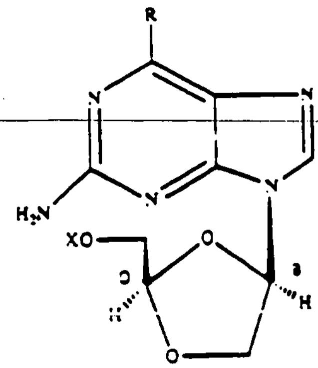
In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such fomulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent N . 4,522,811

be prepared, for example, by reacting the nucleoside with tosyl chloride in pyridine at room temperature for about 24 hours, working up the product in the usual manner (e.g., by washing, drying, and crystallizing it).

The triphosphate can be prepared according to the procedure of Hoard et al., J. Am. Chem. Soc., 87(8), 1785-1788 (1965). For example, ß-D-dioxolane-nucleoside is activated (by making a imidazolide, according to methods known to those skilled in the art) and treating with tributyl ammonium pyrophosphate in DMF. The reaction gives primarily the triphosphate of the nucleoside, with some unreacted monophosphate and some diphosphate. Purification by anion exchange chromatography of a DEAE column is followed by isolation of the triphosphate, e.g., as the tetrasodium salt.

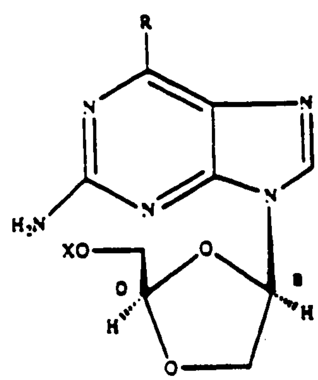
This invention has been described with referenc to its preferred embodiments. Variations and modifications of the invention, enantiomerically pure ß-D-dioxolane-nucleosides, will be obvious to those skilled in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications be included within the scope of the appended claims.

3. A method for the treatment f HBV infection in a human or other host animal, comprising administering an HBV treatment amount of an enantiomerically pure ß-D-dioxolanyl nucleoside of the structure:



wherein R is H or Cl, and X is selected from the group consisting of alkyl, acyl, monophosphate, diphosphate, and triphosphate, or its pharmaceutically acceptable salt, and wherein the compound is 95% free of the corresponding ß-L enantiomer.

4. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the racemic mixture of a ß-dioxolanyl nucleoside of the structure:



wherein R is H or Cl, and X is selected tr m the group consisting of alkyl, acyl, monophosphate, diphosphat , and triph sphate, or its pharmaceutically acceptable salt.

7. The method of claims 1, 2, 3, 4, 5, or 6 wherein the carrier is suitable for oral delivery.

8. The method of claims 1, 2, 3, 4, 5, or 6 wherein the carrier comprises a capsule.

9. The method of claims 1, 2, 3, 4, 5, or 6 wherein the carrier is in the form of a tablet.

10. The method of claims 1, 2, 3, 4, 5, or 6 wherein the administration is parenteral.

11. The method of claims 1, 2, 3, 4, 5, or 6, wherein the alkyl group is selected from the group consisting of methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, t-butyl, isopentyl, amyl, t-pentyl, cyclopentyl, and cyclohexyl.

12. The method of claims 1, 2, 3, 4, 5, or 6, wherein the acyl group is selected from the group consisting of acetyl, propionyl, butyryl, pentanoyl, 3-methylbutyryl, hydrogen succinate, 3-chlorobenzoate, benzoyl, acetyl, pivaloyl, mesylate, propionyl, valeryl, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic.

13. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 1 in alternative dosages with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2′-fluoro-5-iod -arabinosyluracil (FIAU); an

oxathi lane; an enantiomer or racemic mixture of 2'-flu ro-5-iodo-arabinosyluracil (FIAU) ; an enantiomer or racemic mixture of 2'-fluor -5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

17. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 5 in alternative dosages with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2'-fluoro-5-iodo-arabinosyluracil (FIAU) ; an enantiomer or racemic mixture of 2'-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

18. A method for the tre.tment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 6 in alternative dosages with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-I,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2'-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2'-fluorc-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

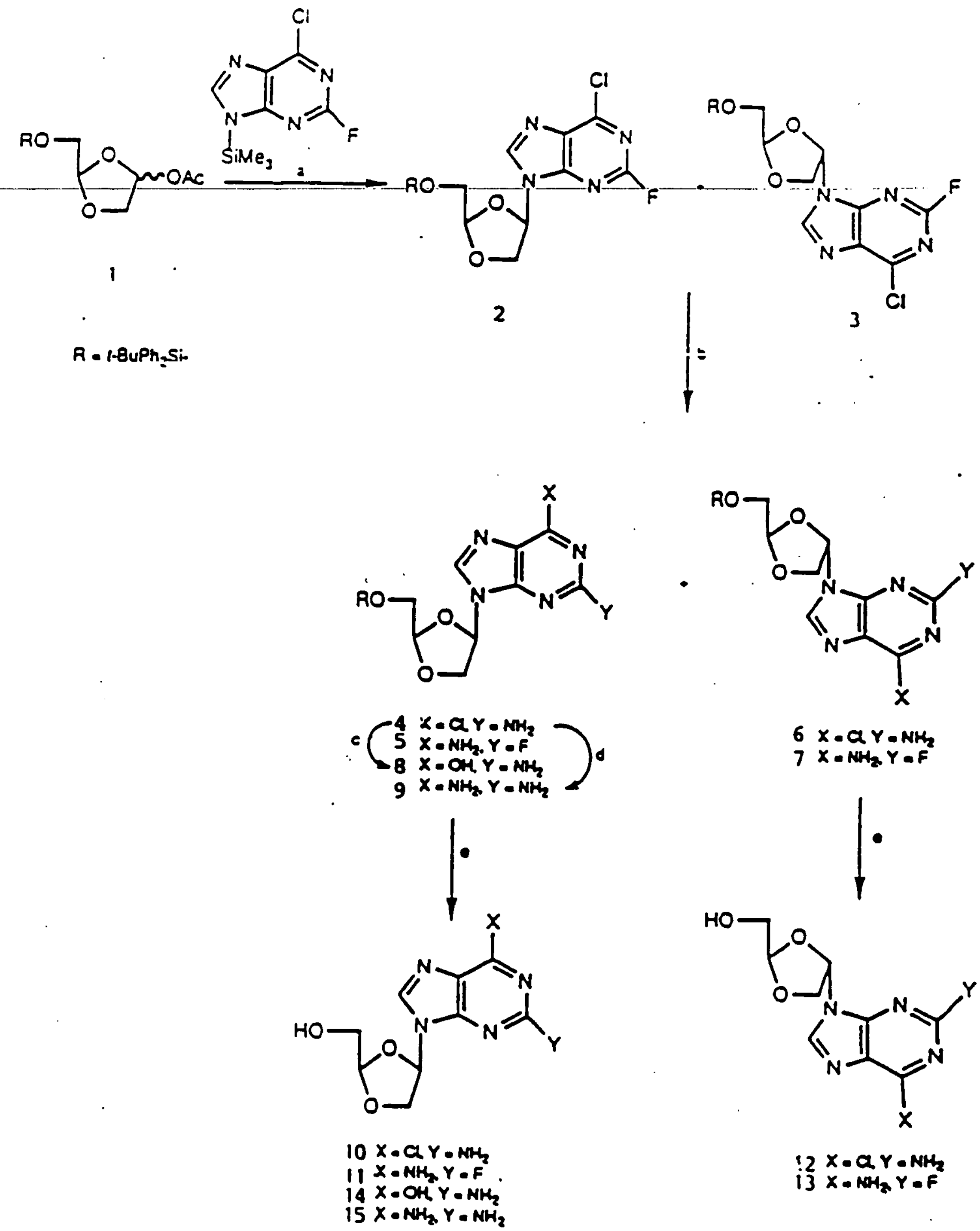
19. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 1 in combination with a compound selected from the group c nsisting f the

22. A meth d for the treatment of HIBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 4 in combination with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-I,3-oxathiolane; an enantiomer or racemic mixture of 2'-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2'-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

23. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HBV treatment amount of the nucleoside of claim 5 in combination with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-I,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cytosin-1-yl)-1,3-oxathiolane; an enantiomer or racemic mixture of 2'-fluoro-5-iodo-arabinosyluracil (FIAU); an enantiomer or racemic mixture of 2'-fluoro-5-ethyl-arabinosyluracil (FEAU), carbovir, or interferon.

24. A method for the treatment of HBV infection in a human or other host animal, comprising administering an HAV treatment amount of the nucleoside of claim 6 in combination with a compound selected from the group consisting of the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-oxathiolane; the (-)-enantiomer or racemic mixture of 2-hydroxymethyl-5-(cyt sin-1-yl)-1,3-

Figure 1



[1]Reagents: (a) TMSOTf, CH_2Cl_2; (b) NH_3, DME; (c) $HSCH_2CH_2OH$, NaOMe; (d) NH_3, EtOH; (e) n-Bu_4NF, THF.

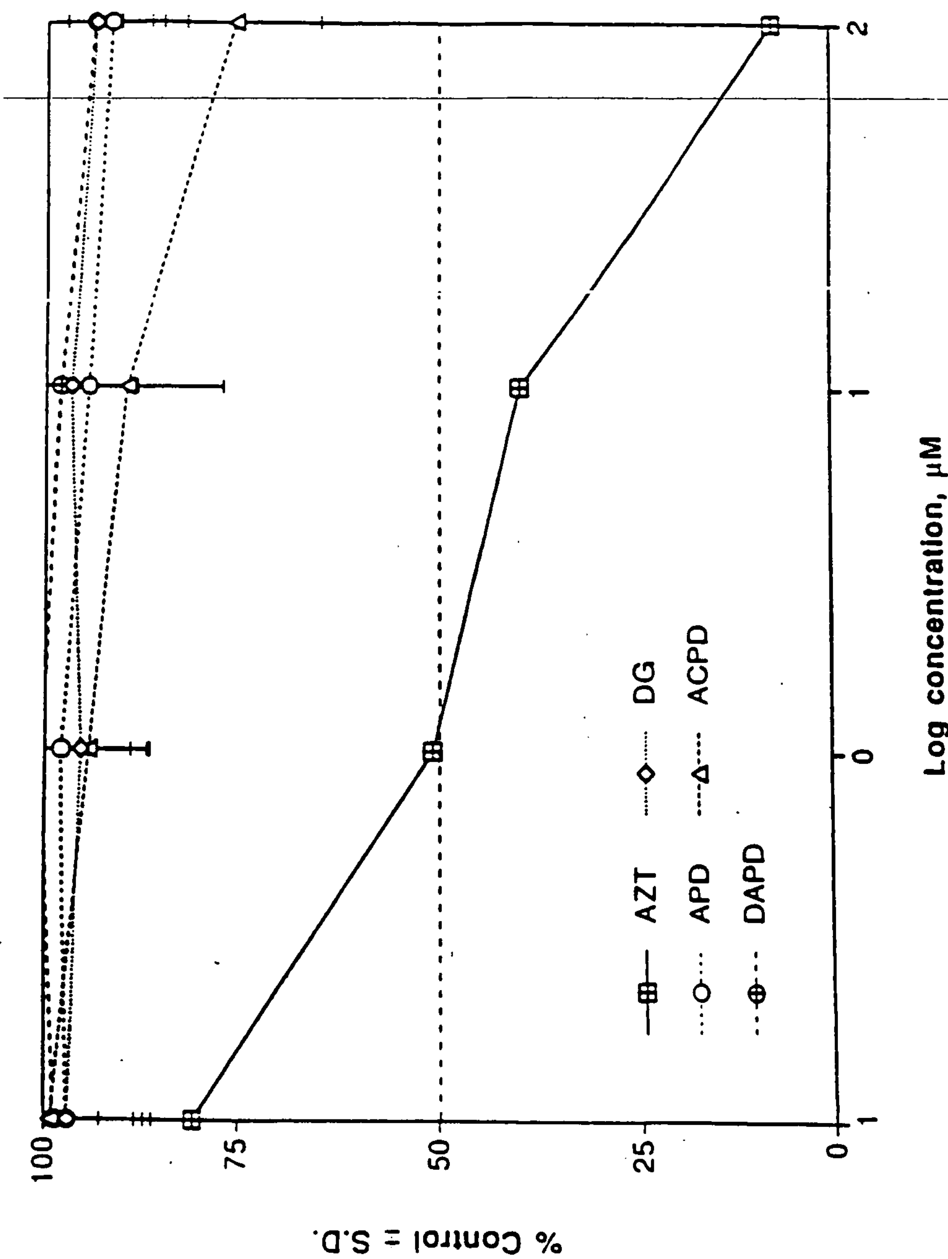

AZT
DG
APD
ACPD
DAPD
% Control ± S.D.
100
75
50
25
0
-1
0
1
2
Log concentration, μM

Figure 2

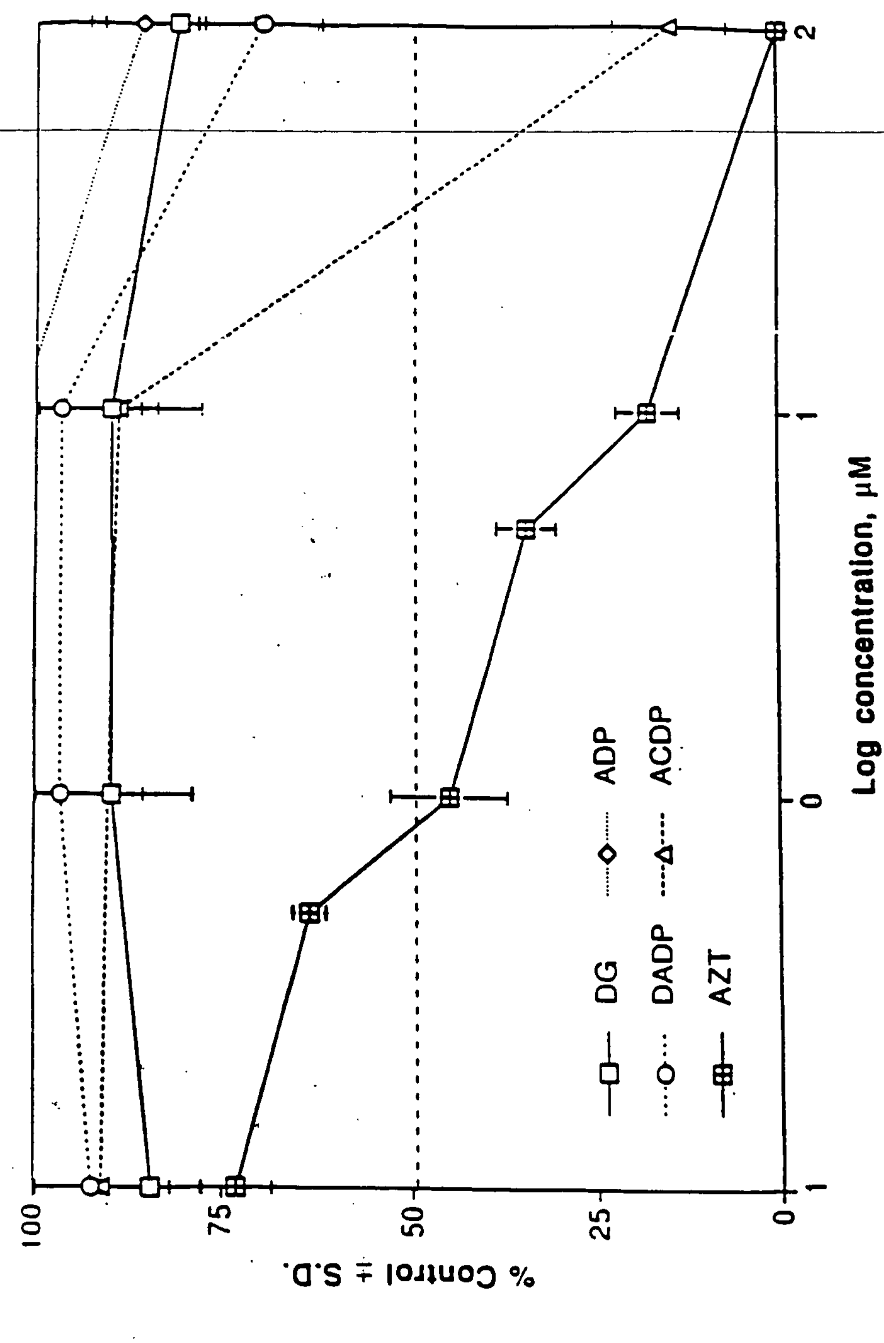

% Control ± S.D.
100
75
50
25
0
Log concentration, µM
-1
0
1
2
DG
DADP
AZT
ADP
ACDP

Figure 3

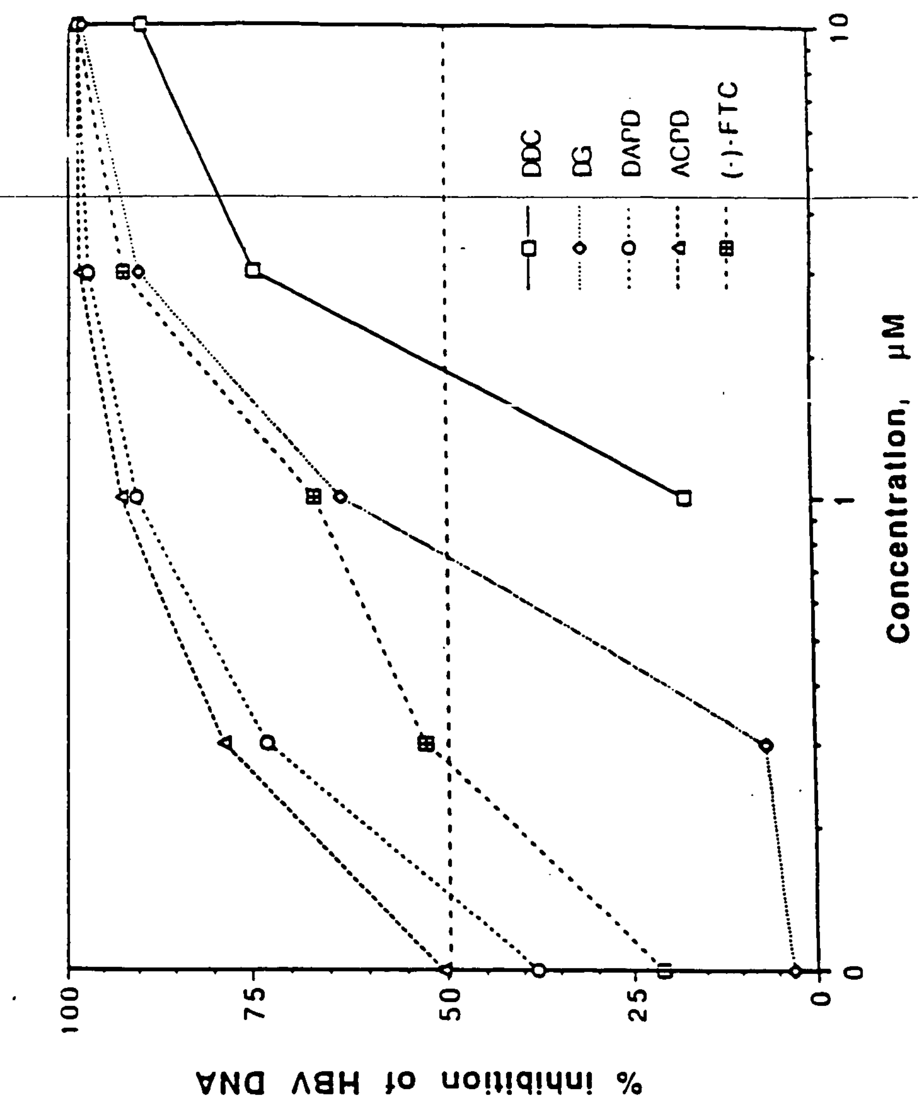

DDC
DG
DAPD
ACPD
(-)-FTC
% inhibition of HBV DNA
100
75
50
25
0
Concentration, µM
0
1
10

Figure 4

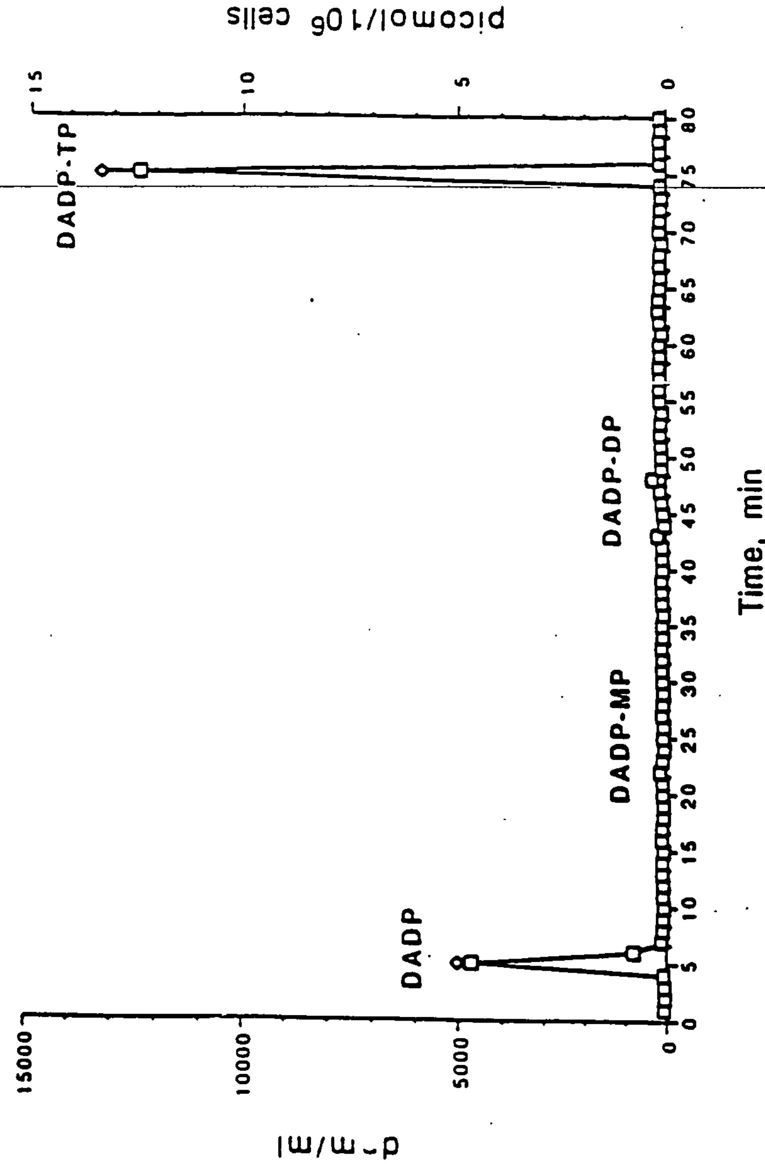

picomol/10^6 cells
dpm/ml
Time, min
DADP
DADP-MP
DADP-DP
DADP-TP
0
5
10
15
5000
10000
15000
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80

Figure 5

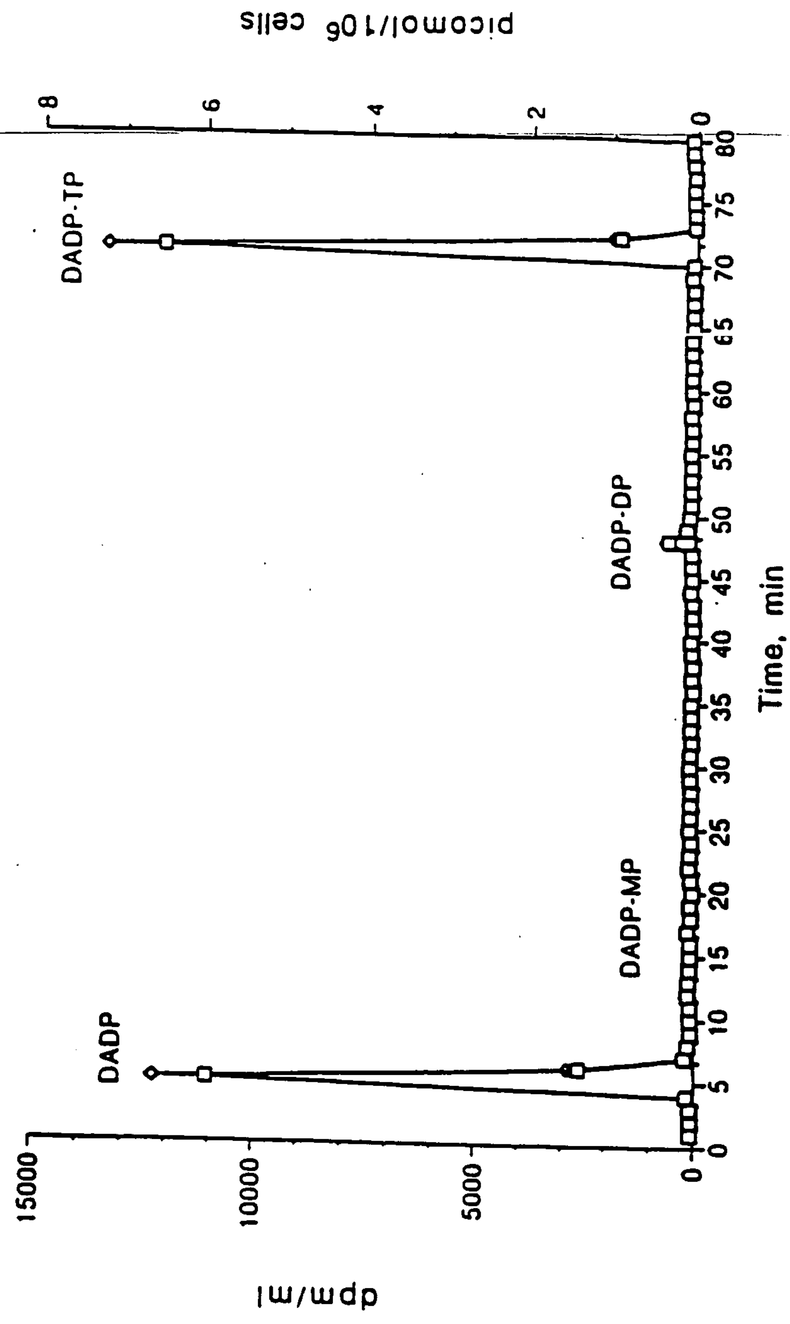

picomol/10^6 cells
8
6
4
2
0
DADP-TP
DADP-DP
DADP-MP
DADP
15000
10000
5000
0
dpm/ml
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
Time, min

Figure 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 93/10348

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 337 713 (IAF BIOCHEM INTERNATIONAL INC.) 18 October 1989 see the whole document ---	1-24
Y	WO,A,92 10497 (UNIVERSITY OF GEORGIA RESEARCH FOUNDATION) 25 June 1992 cited in the application see abstract; claims ---	1-24
Y	WO,A,92 08717 (IAF BIOCHEM INTERNATIONAL INC.) 29 May 1992 see abstract see page 40, line 35 - page 41, line 24; claims 1-17; examples 18-21 --- -/--	1-24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
19 January 1994	11.02.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hoff, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATI NAL SEARCH REPORT

Inter. ıonal application No.
PCT/US93/10348

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 1-24 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.